Hypoxia and iodoacetic acid and alveolocapillary barrier permeability to albumin

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The exact threshold of tolerance of biological membranes to oxygen deprivation as to effects upon protein is still controversial. Landis (8) showed that in the virtual absence of oxygen the capillaries of the frog mesentery become permeable to protein. This observation has frequently been extrapolated to suggest that milder degrees of hypoxia may promote edema formation in various organs by increasing permeability to plasma protein. Maurer (11) showed in dogs breathing 10% O2, whose arterial oxygen content was 6.5 vol %, that right thoracic lymph flow increased to 4 times the level when breathing room air. Warren and Drinker (20) also observed increased lymphatic flow when dogs breathed 8.6-10% oxygen mixtures and concluded from this that marked pulmonary hypoxia causes increased capillary permeability which promotes pulmonary edema. These deductions, based on increased lymph flow, have been challenged (2, 5, 6, 10), but permeability changes cannot be ruled out on the basis of published research as a contributing cause of pulmonary edema, since no critical studies of lung alveolocapillary membrane permeability under conditions of hypoxia have been published. The studies of Landis (8) dealt with virtual anoxia in mesenteric capillaries, whereas interest in effects of hypoxia in lung edema production is concerned with varying degrees of hypoxia upon a different membrane structure.

Previous work (1) indicates that severe hypoxia did not aggravate pulmonary edema produced by other means. This paper deals with a quantitative study of the effects of hypoxia and a metabolic poison on the permeability of the alveolocapillary membrane to plasma albumin in isolated perfused dog lungs.

METHODS AND MATERIALS

Preparation of lungs. A modification of the isolated perfusion technique described by Taylor et al. (19) was used. Adult mongrel dogs were anesthetized with sodium pentobarbital 30 mg/kg iv and heparinized systemically with 10 ml heparin 1:1,000 iv. The dogs were ventilated with 100% O2 for 15-30 min to eliminate dissolved N2. The trachea was then occluded, and a pneumothorax was created to allow atelectasis to occur as the O2 and CO2 were absorbed by the blood. The dogs were exsanguinated, and the plasma was used for isolated perfusion. After cardiac arrest, the heart and the degassed lungs were immediately removed and the left lower lobe was isolated from the heart and other lobes by crushing ligatures of umbilical tape. These other structures were excised. The left lower lobe with its attached bronchus and segment of trachea was weighed (34-67 g), and the left main pulmonary artery and lower lobe vein were cannulated, care being taken not to introduce air bubbles into the arterial side. The vascular perfusion system included plasma-filled connecting tubing, a small bubble oxygenator, finger perfusion pump, and bubble trap on the arterial side (see Fig. 1). The trachea was cannulated and the lobe was immersed in a saline-filled plethysmographic container at room temperature (22 ± 2 C). A short trial of vascular perfusion was begun because initial adjustments in the angulation in the cannulas were needed to reduce arterial perfusion pressures to below 15 mm Hg. Then the perfusion was stopped and the tracheoalveolar system was filled with Tyrode solution labeled with approximately 30 μc of human serum albumin-131. Inorganic 131I was largely removed from the albumin solution prior to use by dialysis against cold-buffered saline at pH 7.4 with two or three changes for 24 hr and represented less than 0.001% of the total bound iodine.
However, because of the large difference in permeability of the biological membrane to I\(^-\) and to albumin, the traces of I\(^-\) resulted in larger rates of total \(^{131}\)I movement over the first few minutes. Another factor also had to be taken into account, namely that the extravascular extracellular fluid, as well as the residual capillary fluid, was exposed for some minutes to zero vascular flow while diffusion was occurring. Consequently, the first minutes of perfusion carried away extra quantities of albumin-\(^{131}\)I.

The possibility that the iodine in albumin-\(^{131}\)I might be split from the protein in the course of membrane passage or by the blood itself was tested. It was found that during the course of 2 hr while blood levels of \(^{131}\)I rose from 0 to 438 counts/min per milliliter of blood, after trichloracetic acid precipitation of the protein in the blood the supernatant showed only 19 counts/ml blood per minute above background. Since this value is very small, little larger than the counting error, \(\pm 16\) counts/min, relative to the protein-bound \(^{131}\)I, no correction has been made for it in the data reported. The further theoretical possibility exists that inorganic I\(^+\) in plasma might combine with protein and thus introduce a complication which would invalidate the assumption that albumin \(^{131}\)I found in the perfusate had passed the alveolo-capillary barrier in the bound form. Schultz et al. (17) tested this equation and found that in the absence of the thyroid gland no measurable binding occurred in 6 hr in otherwise intact dogs. They also showed that with in vivo tagging 0.2\% or less of inorganic I\(^+\) was bound in 6 hr. Two-tenths percent of the inorganic I\(^+\) is a negligible fraction for the purposes of this study.

**Measurements and sampling procedures.** Measurements of alveolar volume (Va), vascular volume (Vb), lung weight, and rates of movement of the test substance were made in order to calculate the permeability coefficient (\(p\)) as described later. The volume change due to the filling of the alveolobronchial compartment of the degassed and unperfused lobe of the lung was measured by the displacement of saline from the plethysmograph as the lobe was filled with Tyrode solution. The volume displacement was taken as equal to the alveolar volume (Va), since it was assumed that the more rigid trachea and bronchi did not change volume. Agreement of this method with that of washout curve analysis described by Taylor et al. (19) was within 10%.

The pulmonary artery pressure averaged 13 mm Hg. No sustained increase occurred with hypoxia. The venous outflow pressure was set at zero at the level of the exit from the top of the plethysmograph. The transmural pressures at different heights of lung in the chamber were therefore determined solely by the resistances in the vascular bed, since the hydrostatic column effect was cancelled out by the fact that the chamber was filled with saline. Inspection indicated that the entire lung lobe was inflated with fluid and was being perfused.

The vascular volume (Vb) included the measured volume of the plasma introduced into the perfusion circuit plus an estimated 3 ml of blood in the degassed lungs. The interstitial fluid volume was ignored in the calculations, since it was part of the alveolo-capillary barrier.

In order to estimate surface area (\(A\)), expressed in square centimeters, the area-to-weight conversion ratio, \(A = 0.661\) W, was used, based on Taylor's interpretation of data from Roughton (16) and Miller (12) on human lungs. Wet weight of degassed lungs (W) was expressed in grams. It is assumed that there is uniform perfusion of all capillaries. Microscopic studies of perfused lungs indicate that very few capillaries have not been perfused, as indicated by the fact that erythrocytes, which fill capillaries which have not been perfused, were rarely found in the lungs perfused in this study.

**Fig. 1.** Isolated perfused dog lung. A: lung lobe is placed in a saline-filled plethysmograph. B: blood recirculates at a constant flow rate through tubing which includes a gas bubbler. C: tracheal lavage fluid enters alveolar system through valve on tracheal line. Arterial, venous, and tracheal tubes are connected to Statham strain gauge pressure transducers for continuous pressure monitoring.
A detailed derivation is given by Riggs (14), and analogous equations can be found in the papers of Robertson (15) and Solomon (18). Crucial to its correct application here are the two major assumptions that: 1) under these flow rates albumin transfer is diffusion limited at the alveolo-capillary barrier, and 2) the lung behaves like a homogenous two-compartment system for albumin, i.e., mixing in each is essentially instantaneous. Support for the first assumption comes from the fact that the vascular fluid perfusate concentration of isotopes rose by less than 0.01% of the alveolar fluid concentration per circuit of fluid; consequently flow limitation is ruled out.

As to the second assumption, calculations of the free diffusion of albumin within the liquid-filled alveolus indicated that the alveolocapillary barrier imposed the major restriction to transport. Using the diffusion coefficient for albumin in water given by Landis and Pappenheimer (9), one calculates that free diffusion of albumin along a mean path length equal to the assumed alveolar diameter (200 μ) is 850 times more rapid than transport across the alveolocapillary membrane as measured in our studies.

Other minor assumptions are made, such as that the absorption of water from alveoli is small. Absorption of 10% (the maximum observed over 5 hr) would change permeability values by less than 5%.

It should be noted that if a smaller average alveolar diameter were to be assumed the calculated area for exchange which is not available. The latter would require precise knowledge of areas for exchange which is not available. The p values presented are, however, believed to be correct as to order of magnitude.

Effect of hypoxia upon blood appearance rates of labeled albumin. The rate of albumin transfer from the alveolus into the vascular compartment was compared in oxygenated and hypoxic lungs in which each lung served as its own control. The vascular perfusate was first gassed with 95% N₂ and 5% CO₂ for 60–90 min, followed by 95% O₂ and 5% CO₂ for 60–90 min. A portion of the plasma was removed and replaced by oxygenated plasma at the time of the shift. In one dog the sequence was reversed.

### Table 1. Tyrode solution containing ^125^I-labeled albumin placed in alveolar compartment

<table>
<thead>
<tr>
<th>Dog</th>
<th>Va, ml</th>
<th>Vb, ml</th>
<th>Oxygenated</th>
<th>Hypoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>χ 10^3 cm²</td>
<td>χ 10^3 sec</td>
<td>χ 10^3 cm/sec</td>
<td>Tension, mm Hg</td>
</tr>
<tr>
<td>8</td>
<td>112</td>
<td>2.01</td>
<td>5.64</td>
<td>2.09</td>
</tr>
<tr>
<td>10</td>
<td>103</td>
<td>2.21</td>
<td>4.48</td>
<td>3.36</td>
</tr>
<tr>
<td>11</td>
<td>168</td>
<td>4.18</td>
<td>5.66</td>
<td>1.86</td>
</tr>
<tr>
<td>12</td>
<td>280</td>
<td>3.26</td>
<td>6.18</td>
<td>3.01</td>
</tr>
<tr>
<td>Avg</td>
<td>103</td>
<td>3.62</td>
<td>4.92</td>
<td>2.86</td>
</tr>
</tbody>
</table>

Oxygenated and hypoxic conditions compared in each lung. Permeabilities (p) calculated from the appearance rates of isotopes in vascular compartment.
**ALVEOLOCAPILLARY PERMEABILITY**

**Effect of iodoacetate on blood appearance rate of labeled albumin.** In four experiments hypoxic perfusion was established with 95% nitrogen, 5% CO₂ for 60–90 min, and then sodium iodoacetate solution was added to the vascular compartment to bring its concentration to 2 mM. In one of these experiments the hypoxic perfusion was preceded by oxygenated perfusion.

**RESULTS**

**Effect of hypoxia.** Figure 2 shows in the upper two graphs the results of experiments where isotopic equilibration slopes are compared under hypoxic and oxygenated conditions. Table 1 presents the data for four such experiments in which hypoxia was maintained for 2–3 hr. In oxygenated controls, the average permeability coefficient was 2.86 $\times$ 10⁻⁹ cm sec⁻¹. The average for the hypoxic state was 2.56 $\times$ 10⁻⁹ cm sec⁻¹. In the latter condition three of the four lungs showed slightly lower permeability in the hypoxic state. The average oxygen tension during hypoxia in these cases was 19.6 mm Hg. In the fourth dog, middle graph of Fig. 2, a small rise of permeability from 3.01 $\times$ 10⁻⁹ to 4.16 $\times$ 10⁻⁹ cm sec⁻¹ occurred at 1 mm Hg O₂ tension. However, the slope of the disappearance line was linear. There was no evidence of a progressive change due to the increasing hypoxia.

**Effect of iodoacetate poisoning.** In the second group of experiments, hypoxia was induced and permeability measurements were made. Figure 2 (lowest graph) shows the effects of the addition of 2 mM iodoacetate. The effect of iodoacetate on isotopic appearance rate became detectable after 22 min in these experiments. Table 2 shows an average increase in permeability from 3.64 to 132 $\times$ 10⁻³ cm sec⁻¹, which is a 36 fold difference. Statistical treatment of the data by t test of the regression slopes shows that the probability is <0.001 that the observed differences in rates of ¹³¹I appearance in the control and iodoacetate periods could have occurred by chance.

**DISCUSSION**

The continuing linearity of the relation between the log of vascular compartment concentration over time and the lack of significant change in albumin appearance rates when the hypoxic state is compared with the oxygenated state suggest that hypoxia of the degrees and duration employed does not appear to alter the permeability of the dog alveolocapillary barrier under conditions of controlled constant perfusion rate.

Nevertheless, the integrity of the membrane is apparently not independent of metabolic processes, since iodoacetate, which alkylates glyceraldehyde phosphate dehydrogenase irreversibly, was found to cause a profound increase in permeability to albumin. In the experiments reported here the iodoacetate was administered during exposure to hypoxia. It may be noted that it has been found (unpublished data) that the edemogenic effects of this agent occurred at the same dose levels in the oxygenated and hypoxic lung.

The work of Nicoloff et al. (19) studying weight changes in isolated dog lungs perfused with blood at 37°C did not show a detectable increase in edema production under controlled perfusion conditions when lung ventilation was shifted from normal O₂ and CO₂ tensions to 95% N₂ + 5% CO₂. They measured the rates of edema formation at pulmonary artery pressures elevated above the threshold for edemogenesis, induced by increasing the perfusion rate. When there was a shift from a ventilation gas mixture of 20% O₂ + 75% N₂ + 5% CO₂ to 95% N₂ + 5% CO₂ for periods of from 8 to 38 min, there was no increase in the rate of gain in lung weight at identical perfusion rates.

It must be emphasized that the absolute values for permeabilities are subject to whatever error there may be in calculations of surface area and also in extrapolation of appearance rates to the half-equilibrium time. The relation between lung weight and capillary surface area is based upon measurements in the human lung. It is known that the alveolus in the dog lung has a smaller diameter than in the human. Therefore, there may be a systematic error introduced by this factor. Another factor entering into the area value is the completeness of perfusion of the entire capillary bed. We have assumed uniform perfusion and to the extent this may be in error the absolute values for p would be affected correspondingly.

The changes in calculated values for p from the control to the experimental conditions are more significant, although they too might occur due to alterations in the distribution of perfusate, such as the possible opening of previously closed capillaries, or vice versa, when hypoxia was imposed or iodoacetate was administered. To minimize such effects, the pulmonary venous pressure was maintained at essentially zero with reference to atmospheric throughout experiments.

Large shifts in water between Va and Vb over the course of an experiment would also affect the calculated results. A measurable absorption of water from the alveolar-to-vascular compartment does occur under the conditions employed, but the magnitude of the shift was such that the error in the calculated permeabilities was small. Furthermore the water flux into the vascular compartment can probably be assumed to be at a constant rate, since it is due to the colloid osmotic pressure of the plasma and the absence of colloid in the alveolar fluid, and there were minimal transmural hydrostatic pressure changes.

**Table 2. Tyrode solution containing albumin ¹³¹I placed in alveolar compartment under hypoxic conditions**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Va, ml</th>
<th>Vb, ml</th>
<th>A</th>
<th>P₀₁</th>
<th>t₁</th>
<th>P₁</th>
<th>Dura-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm Hg</td>
<td>10⁻⁵ cm²</td>
<td>sec</td>
<td>cm/sec</td>
<td>sec</td>
<td>cm/sec</td>
<td>Poison, mI</td>
</tr>
<tr>
<td>12</td>
<td>129</td>
<td>280</td>
<td>3.26</td>
<td>1.47</td>
<td>4.16</td>
<td>0.452</td>
<td>41.0</td>
</tr>
<tr>
<td>13</td>
<td>131</td>
<td>176</td>
<td>3.40</td>
<td>2.21</td>
<td>6.74</td>
<td>0.255</td>
<td>58.5</td>
</tr>
<tr>
<td>14</td>
<td>111</td>
<td>168</td>
<td>2.71</td>
<td>8.26</td>
<td>2.07</td>
<td>0.148</td>
<td>115.0</td>
</tr>
<tr>
<td>15</td>
<td>121</td>
<td>174</td>
<td>3.27</td>
<td>5.94</td>
<td>1.60</td>
<td>0.0484</td>
<td>313.0</td>
</tr>
<tr>
<td>Avg</td>
<td>123</td>
<td>199</td>
<td>3.18</td>
<td>7</td>
<td>6.098</td>
<td>3.64</td>
<td>0.226</td>
</tr>
</tbody>
</table>

Effect of addition of sodium iodoacetate 2 mM noted. Permeability (p) calculated from appearance rates (t₁) in the vascular compartment.
Since the lungs in these experiments were exposed to hypoxia during the "degassing" preparation period, it might be thought that an irreversible increase in permeability occurred which would obscure an effect of induced hypoxia during the experiment. That this did not occur can be concluded from the fact that the lungs did not become edematous at perfusion pressures of 13 mm Hg and from the low permeability constants for albumin which were calculated from the flux data. Furthermore, the greatly increased permeability to albumin produced by iodoacetic acid reported in this paper, and also the large increase in protein permeability related to dose induced by alloxan, which is an agent inducing acute pulmonary edema, as reported by Goetzman and Visscher (4), demonstrate that the alveolocapillary membrane can become permeable to albumin under conditions in which acute lung edema does occur.

It should be noted that the studies on albumin flux reported in this paper were performed at room temperature. Oxygen requirements for lung tissue are reduced, and it might be suggested that the failure to find an increase in permeability to albumin with hypoxia might be due to this situation. However, it may be noted that the studies of hypoxia and edemogenesis reported by Nicoloff et al. (13) were performed at 37 C. Those studies showed no edemogenic effect of comparable levels of hypoxia in the isolated dog lung perfused with blood.

The conclusions of Maurer (11) and Drinker (3) that relatively mild hypoxia increases permeability to protein in lung capillaries and thus promotes pulmonary edema have not been corroborated. There has been a renewed interest in this problem because of the suggestion that high-altitude acute pulmonary edema in man might be a consequence of increased pulmonary capillary permeability to protein in hypoxia. The considerations in relation to this problem have been reviewed by Hultgren et al. (7). The data presented in this paper do not support this possibility, since oxygen tensions lower than could be compatible with life did not increase the permeability of the alveolocapillary barrier.

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